

The Use of Ultrasound to Control Algae at Sewage Lagoons in Ontario to Meet Environmental Compliance Approvals

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Introduction

Cyanobacteria, commonly called blue-green algae, are microscopic organisms that have inhabited the earth for over 2 billion years. Although they are bacteria, they have features in common with algae and occur naturally in a wide variety of environments including ponds, rivers, lakes, water reservoirs and frequently, in sewage lagoons. Blue-green algae are very noticeable and generally become a concern when growth occurs exponentially resulting in the appearance of a bloom on the surface of the water. Wind and currents can concentrate the algae into sheltered areas resulting in the appearance of a scum. Blooms are reported most commonly during the summer season, usually in association with warm water temperatures and calm conditions that provide an optimal environment for reproduction; but blooms often occur into the fall and can occur under the ice if light is able to penetrate (i.e. clear ice and no snow cover). The presence of phosphorus (P) at substantial concentrations (i.e. eutrophic lakes) are often associated with blue-green algae blooms but blooms also occur in oligotrophic lakes (Carey *et. al.* 2012) with microcystin concentrations correlated strongly with cellular protein content which was highest at medium when nitrogen (N) is not limiting as indicated by N:P ratios between 18 and 51 (Downing *et. al.*, 2005). Thus, it is evident that P alone is not the sole determinant of microcystin blooms.

Sewage lagoons provide an ideal environment for blue-green algae growth in that they have high N and P concentrations, are relatively shallow, have a small surface area which limits wave action, designed to have a controlled residence time and are generally warmer and ice free for longer periods than natural ponds. Most lagoons, however, have aeration systems to ensure circulation and oxygenation. The turbulence caused by the aeration systems may reduce algae growth, especially blue-green algae because these algae optimize growth by controlling their buoyancy with the gas vesicles within their cell.

Cyanobacteria have been demonstrated by Others have demonstrated the effectiveness of ultrasound to effectively control cyanobacteria. See for example Rajasekhar *et. al.*, 2012; Zhang *et. al.*, 2005; Song *et. al.*, 2005; and Lee *et. al.*, 2002). A frequent concern with the use of ultrasound is that the ultrasound vibration would result in cell lysing (breaking of the cell wall) which could release the microcystin toxins stored within the blue-green algae cell. As with demonstrations elsewhere, this ultrasound does not result in cavitation; and consequently, does not lyse the blue-green algae cells. This will be considered further below.

Demonstration Sites

Sundridge, located approximately 50 km north of Huntsville, ON, has two lagoons. The main, active lagoon is aerated with a series of fine bubble diffusers extending across the lagoon at depth as illustrated in Figure 1. Further, the lagoon is subdivided into three sections by two impermeable geomembrane flow diversion baffles with limited openings or windows for maximum flow of 0.020 m/sec between each cell. There was a concern that these impermeable flow diversion baffles may interrupt the ultrasonic signal or reduce the effectiveness of the ultrasound that was placed in the middle of the lagoon, approximately mid-way between the two baffles.

The Sundridge sewage lagoon is regulated under an Environmental Compliance Approval (ECA) issued by the Ontario Ministry of Environment, Conservation and Parks (MECP). Frequent exceedances of the TSS limits of the ECA were a concern with algae blooms being the main cause of these exceedances. Algae, also affects the efficiency of the underground Submerged Attached Growth Reactor (SAGR) system that is installed downstream of the lagoon to control ammonia concentrations in the wastewater. A cost-effective solution that would control or eliminate the algae blooms would allow the system to meet its TSS effluent limits of the ECA and directly benefit the operation of the SAGR. Water samples collected on July 14, 2020 confirmed that the algae in the lagoon were

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pre-dominantly the cyanobacteria Chlorophyceae (*Chlamydomonas sp.*) and Cyanophyceae (*Pseudanabaena sp.*)¹. A solar powered ultrasonic unit was installed on August 18, 2020 in the treatment lagoon.

Similarly, MECP sets effluent limits under an ECA for the spring discharge from the Town of Essex sewage lagoons. The Essex northeast lagoons (Figure 1) consist of four lagoons that provide initial and progressive treatment prior to effluent discharge during spring freshet into the Puce Drain. Extremely poor water quality in Cell 4 of the lagoons (see photograph 1) led to a decision to test the effectiveness of the ultrasound to control algal blooms in the cell. The MECP agreed to an extension of the discharge window to May 15, 2021 to allow time for the ultrasound, installed on March 31, 2021, to control algae in the lagoon. A lagoon water sample collected on March 16, 2021 and analysed by ALS demonstrated that the algae in the lagoon were dominated by cyanobacteria consisting of Chlorophyceae (*Chlamydomonas sp.*), Chlorophyceae (Unidentified), Cyanophyceae (*Aphanothece sp.*) and Chlorophyceae (*Chlamydomonas sp.*). The predominant species at both Sundridge and Essex are known to be effectively managed with ultrasonics.

Ultrasound Technology and How It Works

The ultrasound is a patented ultrasonic and biofilm control device that uses two bandwidths with over 2000 ultrasonic frequencies. The unit has four (4) ultrasound sources such that it covers 360 degrees with a radial range of 400 m (50 hectares) for the control of blue-green algae, a range of 150 m (7 hectares) for the control of green and diatom algae and a range of 60 m (1.1 hectares) for aerobic bacteria. The surface area of the Sundridge lagoon is approximately 3.5 hectares (ha) while Cell 4 in Essex is approximately 6 ha, accordingly, all blue-green, green and diatom algae theoretically will be treated throughout the treatment lagoon along with aerobic bacteria within a portion of each lagoon. The treatment of aerobic bacteria is important for the application in sewage lagoons in that it minimizes the colonization of the transducer heads which would impair its effectiveness and require frequent cleaning. This allowed the ultrasound units to operate for the entire period of the demonstration (2 months at Sundridge and five months at Essex) without maintenance of the in-water unit. As there was no AC power supply directly available at either location, both sites were DC powered (24 V) systems with 200 W solar panels and two 12 V Group 27 Absorbed Glass Mat (AGM) batteries (AGM batteries reduce maintenance as they can tolerate two times more charge/discharge cycles and have a faster recharge rate than conventional flooded batteries). A Morningstar Corporation ProStar 15 amp, 12/24 V solar charge controller was used to manage the solar panels and protect the batteries. The DC power source located onshore provided power to the ultrasound head via a submerged 125 or 150 m cable.

Ultrasonic transducers produce sound waves that occur above the range of human hearing (Figure 2). Ultrasound requires a dense medium such as water or a gel (if used therapeutically on humans) for transmission. Ultrasound technology is widely used in fish finders and depth sounders and the medical field including physiotherapy and diagnostics (foetal echo sounds) or treatment (to break up kidney stones). The use of the ultrasound determines the “power” of the source. The has been tested extensively in our own facility and has a peak pulsed power demand of less than 3 amps at 24 V. At this low power output, it has no visible vibration effect, even in small water containers, thus not inducing cavitation. While controlling a large number of algae species, ultrasound does not control weeds and filamentous forms of algae that look more like vegetation due to their structure. Extensive literature on the application of ultrasound to control algae is available in the cited references.

With respect to blue-green algae, ultrasound does not result in lysing (breaking apart) of the cell wall, which is a concern as lysing releases the toxins with the cell. Rather ultrasound stops the growth and reproduction of the algae by disrupting the integrity of the internal gas vesicles that control the vertical movement of the cell. The disruption of the internal gas vesicles of the blue-green algae (see Figure 3) results in the loss of the ability of the algae to

¹ Laboratory counts and species identification for both Sundridge and Essex were conducted by ALS Environmental at their laboratory in Winnipeg, Manitoba. Certificates of analysis are available upon request. ALS is accredited by the Canadian Association for Laboratory Accreditation Inc. (CALA).

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control its movements in the water column. Vertical movement of the cells is necessary to permit access to sunlight and food sources. This loss of buoyancy causes the algae to sink out of the photic zone resulting in a loss of light within approximately 3 to 4 days with overall algae population decay occurring over a period of about several weeks.

Other forms of algae such as green algae are also affected by ultrasound. Through the disruption of the internal cell fluid, green algae will die off in 3 to 4 weeks due to their inability to grow and reproduce. As noted above, the effective range for green algae is more restrictive at a radius of about 150 m.

Anaerobic bacteria are not directly affected by the ultrasound but their growth and colonization is minimized by the ultrasound source by the fact that they sense water turbulence from the ultrasound. The turbulence sensed by the bacteria is neither visible nor sensed by humans and does not detract fish from being in close proximity to the source. As noted, this reduces the maintenance in the form of cleaning that would normally be required in submerged equipment.

Sampling and Analytical Analysis

Water samples were taken periodically at both Sundridge and at Essex throughout the demonstration projects to document conditions and effectiveness of the ultrasound at the site. All samples were collected for the analysis of microcystis (Cyanophyceae) and total cyanobacterial cell count (Algae-Cyanobacteria - blue-green algae) and Microcystin (the measure of the microcystin toxins in the water). Samples were preserved if appropriate and shipped refrigerated to ALS Environmental laboratories in Winnipeg for analysis. The sampling dates for both projects are summarized in Table 1.

The analysis of total microcystin using the Enzyme-Linked Immunosorbent Assay (ELISA) with a de-tetection limit of 0.10 for MECP or 0.20 µg/L for ALS, never exceeded the detection limit on a total of 11 samples. MECP further noted, based on their analysis of a sample from Cell 4 final effluent collected on May 4, 2021 that “No algal mat, cyano bloom or algae bloom found ...” and “The sample contained extremely deteriorated material. There were numerous bacterial cells and debris observed in the sample. The sample also contained numerous extremely small algal cells which were not identifiable due to poor condition”

Table 1 Sample Dates and description of sample for Sundridge and Essex Lagoons

Sundridge Sample Date	Description	Essex Sample Date	Description
July 14, 2020	Pre-demonstration algae characterization – total Cyanobacteria, phytoplankton screen and Microcystin toxins	March 16, 2021	Pre-demonstration algae characterization – total Cyanobacteria and total Microcystin toxins
August 19, 2020	Baseline, date of ultrasound install – total cyanobacteria	April 14, 2021	Progress sampling - total Cyanobacteria and total Microcystin toxins
September 20, 2020	Progress sampling – total cyanobacteria	May 3, 2021	Pre-discharge confirmation sampling - total Cyanobacteria and total Microcystin toxins
October 6, 2020	Progress sampling – total cyanobacteria	March 16 to May 4, 2021	Five samples from the lagoon for microcystin toxins with ELISA

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October 28, 2020	Ultrasound removal and project cessation – total cyanobacteria	May 3 to May 12, 2021	Six samples from Puce Drain, receiving waters for Microcystin toxins with ELISA
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Discussion of Results

The results for all sampling events for both Sundridge and Essex Cell # 4 are summarized in Figure 4. The Sundridge figure demonstrates the reduction in cell counts of Microcystis and Total Cyanobacteria, which is comprised mostly of microcystis. The direct effect of the ultrasound resulted in the reduction Total Cyanobacterial cell count by more than 1300 times (from 2,090,000 on August 18 to 1,600 by Oct. 28, 2020). Most of this reduction was achieved in the first 30 days of operation (from 2,090,000 on August 18 to 4,420 by Sept. 21, 2020 or a reduction of almost 500 times). Aphanizomenon (Cyanophyceae) which was not present in the July sample, doubled between start-up in August and the first sample on September 21 but thereafter was significantly controlled with cell counts declining from 2,440 cells/ml in September to 300 and 0 on October 6 and 28, respectively. It is not surprising to see some species increase after the installation of the ultrasound due to a loss of competition for nutrients and light from other dominant algae.

Although it was expected that the overall benefits of the ultrasound would be limited by the presence of the impermeable membranes that ran across the Sundridge lagoon, this turned out not to be the case. The ultra-sound signal could be detected at strength around the entire lagoon including near the outlet structure. The ability of the ultrasound signal to pass through the membranes is likely due to the fact that it is thin, stretched and directly contacted by water on both sides, which allows the signal to propagate through the membrane. This means that a single ultrasound unit is capable of treating this lagoon and others that may have similar membranes effectively.

Photographs of the lagoon looking from the shore on July 14 and October 8, 2020 in Figure 6 clearly show the change in water column transparency that occurred in the Sundridge lagoon over the course of the project.

The Essex Cell 4, dominated by the single species Aphanothece (*Cyanophyceae sp.*) was reduced from over 14 million cells/mL to 9.4 million cells/mL 14 days after the ultrasound was installed and to 3.1 million cells/mL in the thirty-four days following installation. Additional reduction could have been achieved with continued use of the ultrasound; however, the cell effluent was adequate to meet the requirements of the ECA and the discharge had to be completed by May 15, 2021. Importantly, the ultrasonic treatment did not result in measurable Microcystin toxins in the effluent after four weeks of treatment. The MECP Provincial Officer overseeing the site, stated in e-mail correspondence on August 18, 2021 after review of the data from the May 2021 discharge “... *that the ultrasound treatment was effective in preventing a bloom to form downstream in the river*” and “*Based on these positive results from the spring discharge, we support the use of the ultrasound treatment in the lagoons on a permanent basis.*”

In addition to the control of blue-green algae, ultrasound will reduce the concentration of total suspended solids and the pH in sewage effluent. The low base cost combined with low power requirements that can be readily accommodated through on-site solar systems and the minimal maintenance requirements make ultrasonics an excellent choice to effectively manage sewage lagoons and other water resources that are impacted by blue-green algae blooms.

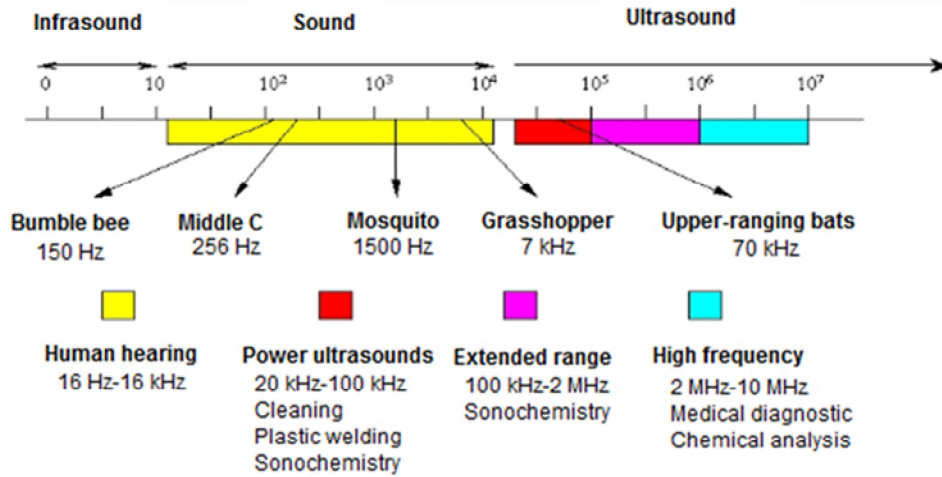
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Photograph 1 Location map of Sundridge and Essex seage lagoons in Ontario

Photograph 2 Sound spectrum illustrating range of ultrasound above the range of human hearing



Photograph 3 Transverse section of a dividing cell of the bacterium (*Microcystis* sp.) showing hexagonal stacking of the cylindrical gas vesicles at x31,500 magnification (Walsby, 1994) and transmission electron microscopy images of ultrasonic damage to vesicles showing loss of gas to outer cell wall which is permeable to the internal gas after 24 hours of exposure (Huang and Zimba, 2020)

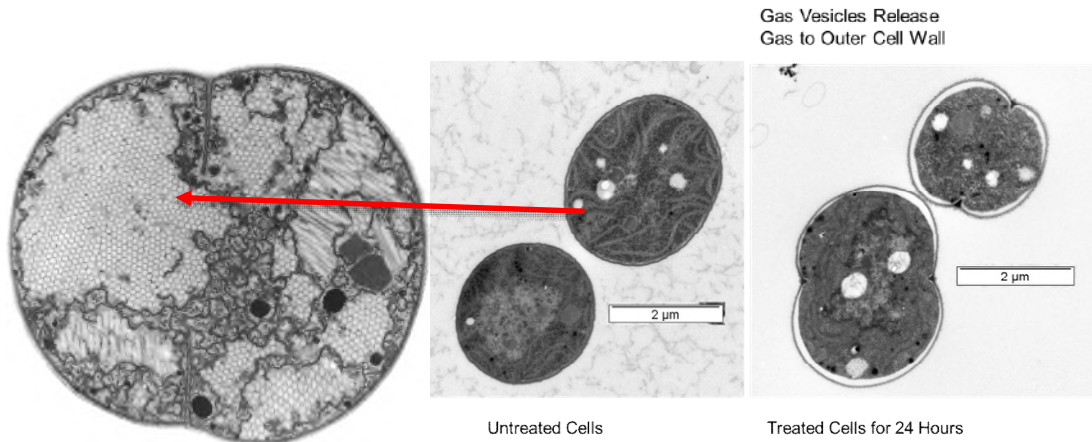
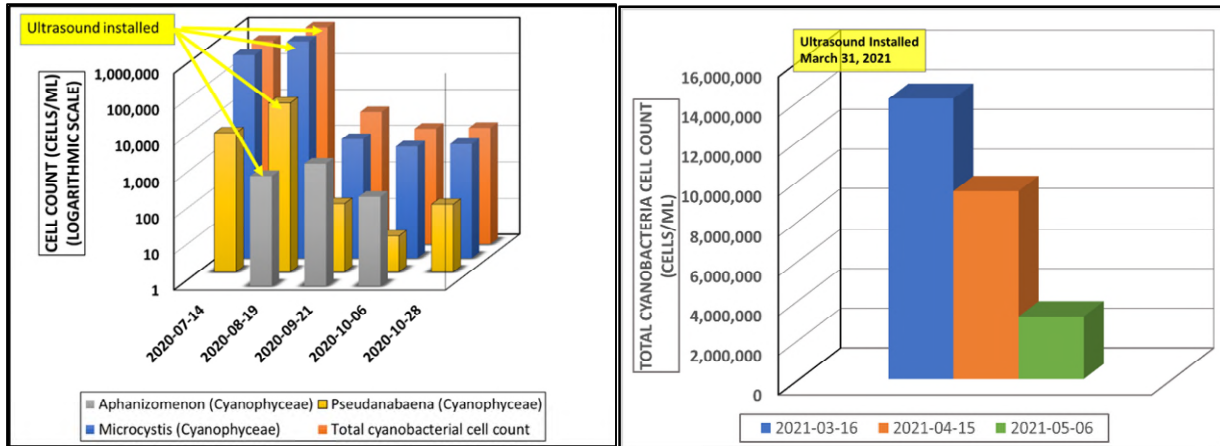


Figure 4

Photograph 4 Cell counts of algal species and total cyanobacteria in Sundridge sewage lagoon in the summer of 2020 with ultra-sound treatment commencing on August 19, 2020 and Total Cyanobacteria count for Essex Cell 4 with ultra-sound treatment commencing on March 31, 2021. A single species, *Aphanothece* (Cyanophyceae) predominated (>98%) in this cell throughout the demonstration and thus only the Total Cyanobacteria count is shown



Photograph 1: Samples of raw water from cell 4, Essex Northeast Lagoons, March 16, 2021.



Photograph 1 Photographs of the Sundridge lagoon on July 14, 2020 (before installation of ultrasound) compared to October 8, 2020, (7 weeks after installation) showing the change in transparency as a result of the control of algae in the lagoon. (October 8, 2020 photo courtesy of J. Richardson)

July 14, 2020



October 8, 2020

